

# The HTD Difference: A deeper look at the HTD equilibrium dialysis system

For over 20 years we have worked closely with scientists at the forefront of the equilibrium dialysis field. In the spirit of discovery, we offer the following reasons to select the HTD system over other industry products for your experimental needs.



#### Time required to reach equilibrium - Separating Marketing Myths vs. Reality

Scientists who have evaluated multiple equilibrium products report that the HTD system reached equilibrium more rapidly than competing systems. The HTD system has a high membrane surface area to sample volume ratio along with low non-specific binding allowing many compounds to reach equilibrium in as little as 4 hours. Investigators at Merck (*see publication*) routinely dialyze for 4 hours.

In our general protocol, a 6-hour incubation time is suggested as this is the most conservative incubation time that allows for setting up the dialysis system, incubation, and sample collection within a standard 8-hour workday. We recommend a simple kinetic experiment with compound spiked into buffer and dialyzed against buffer to evaluate the equilibrium time required (for novel compounds) prior to initiating any binding experiments.



#### **Choosing Construction Materials With Low Non-Specific Binding Properties**

Potential for non-specific binding: The HTD dialysis block is constructed of 100% virgin Teflon which is known to have very low non-specific binding properties for both proteins and test compounds. This is a key difference from competing dialysis systems which are often manufactured using

polystyrene, polypropylene, or polycarbonate which display non-specific binding characteristics.

Challenges associated with non-specific binding: Non-specific binding of test compounds to the various plastics used in competing equilibrium dialysis systems can significantly increase the time required to reach equilibrium for highly bound compounds. Although serum proteins can effectively block many of the non-specific binding sites on the serum side of the dialysis membrane, these blocking agents are not present on the buffer side. As small amounts of the test compound gradually diffuse through the dialysis membrane, they can be continuously absorbed by the plastic non-specific binding sites on the buffer side. For the RED system, the combination of unblocked plastic surfaces and high volume on the buffer side can significantly extend the time for the system to reach equilibrium and distort fraction unbound calculations.





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## Establishing Industry Leading Cost Efficiency Through Reusable, Eco-Friendly Design

The consumable cost for the HTD system is less than \$0.49 per assay. The capital cost of purchasing the reusable HTD system is recovered after only 4-5 experiments when compared to the competing disposable systems. Furthermore, the reusable Teflon block design is more eco-friendly than single use disposable plastic options.

## **Conserving Sample Volumes**

The cost associated with the plasma/serum/tissue and compound used for experiments should be considered as part of the overall experimental costs. The general protocol for the HTD system suggests adding 75-150uL of sample on each side of the dialysis membrane. The RED system suggests adding 300uL to the sample side and 500uL to the buffer side. This significantly increases costs associated with plasma/serum/tissue and compound requirements. In addition, the larger volume required on the buffer side effectively dilutes the compound and increases the time required to reach equilibrium (more compound must cross the membrane before equilibrium can be achieved). This reduces assay sensitivity

## **Minimizing Hands-On Time**

The HTD system was designed to be both setup and disassembled in minutes. This has been consistently confirmed by our user base for over 20 years. The minimal hands-on effort involved in the HTD system is of note as industry competitors often erroneously inflate the setup time in an effort to skew the cost efficiency.

Most manufacturers of dialysis membranes suggest hydrating the membranes for at least an hour before use. We suggest our customers hydrate the dialysis membranes overnight in a solution of buffer plus 10% ethanol. The dialysis membranes are stable stored in the refrigerator and are fully hydrated / ready to use after a quick rinse in the preferred experimental buffer. This approach reduces setup time and ensures that the average pore size of the dialysis membrane has stabilized. A recent publication by Ye and Chen demonstrates the need to precondition/hydrate the RED membranes before use *(Improving the accuracy of unbound fraction measurement of drug-protein binding by preconditioning the RED membrane inserts, Ye and Chen Bioanalysis, vol 12, No.23: 2020).* 

## Achieving Scale Through Automation Compatibility:

The HTD system is fully compatible with automated pipetting decks and 96-well pipetting equipment. Our current customers use systems from a wide range of vendors.



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